PEPTIDE-ENHANCED CATIONIC LIPID TRANSFECTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Continuation-in-Part of U.S. application Ser. No. 08/477,354, filed Jun. 7, 1995, now abandoned, which is incorporated by reference in its entirety herein.

FIELD OF THE INVENTION

Compositions containing peptides, optionally conjugated to DNA-binding groups, and cationic lipids useful for transfecting eukaryotic cells are disclosed. Also disclosed are methods of transfecting eukaryotic cells employing such compositions.

BACKGROUND OF THE INVENTION

Lipid aggregates such as liposomes can function to facilitate introduction of macromolecules, such as DNA, RNA, and proteins, into living cells. Lipid aggregates comprising cationic lipid components can be effective for delivery and introduction of large anionic molecules, such as nucleic acids, into certain types of cells. See Felgner, P. L. and Ringold, G. M. (1989) Nature 337:387–388 and Felgner, P. L. et al. (1987) Proc. Natl. Acad. Sci. U.S.A. 84:7413. Since the membranes of most cells have a net negative charge, anionic molecules, particularly those of high molecular weight, are not readily taken up by cells. Cationic lipids aggregate to and bind polyanions, such as nucleic acids, tending to neutralize the negative charge. The effectiveness of cationic lipids in transfection of nucleic acids into cells is thought to result from an enhanced affinity of cationic lipid-nucleic acid aggregates for cells, as well as the function of the lipophilic components in membrane fusion.

Cationic lipids are not universally effective for transfection of all cell types. Effectiveness of transfection of different cells depends on the particular cationic lipid composition and the type of lipid aggregate formed. In general, polycationic lipids are more efficient than monocationic lipids in transfecting eukaryotic cells. Behr, J-P. et al. (1989) Proc. Natl. Acad. Sci. 86:6982–6986, Hawley-Nelson, P., et al. (1993) FOCUS 15:73 and U.S. Pat. No. 5,334,761 (Gebeyehu et al.). Behr et al. and EPO published application 304 111 (1990), for example, describe improved transfection using carboxyspermine-containing cationic lipids including 5-carboxyspermylglycine dioctadecyl-amide (DOGS) and dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DPPES). Despite their relative effectiveness, however, successful transfection of eukaryotic cell cultures using polycationic lipid reagents requires high dosages of nucleic acid (approximately 10^5 DNA molecules per cell).

Many biological materials are taken up by cells by receptor-mediated endocytosis. See: Pastan and Willingham (1981) Science 214:504–509. This mechanism involves binding of a ligand to a cell-surface receptor, clustering of ligand-bound receptors, and formation of coated pits followed by internalization of the ligands into endosomes. Both enveloped viruses, like influenza virus and alphaviruses, and non-enveloped viruses, like Adenovirus, infect cells via endocytotic mechanisms. See: Pastan, I. et al. (1986) in *Virus Attachment and Entry into Cells*, (Crowell, R. L. and Lonberg-Holm, K., eds.) Am. Soc. Microbiology, Washington, p. 141–146; Kielian, M. and Helenius, A. (1986) "Entry of Alphaviruses" in *The Togaviridae and Flaviviridae*, (Schlesinger, S. and Schlesinger, M. J., eds.) Plenum Press, New York p.91–119; FitzGerald, D. J. P. et al. (1983) Cell 32:607–617.

The introduction of foreign DNA sequences into eukaryotic cells mediated by viral infection is generally orders of magnitude more efficient than transfection with cationic lipid reagents. Viral infection of cell cultures requires fewer than 10 virus particles per cell. Although the detailed mechanism of fusion is not fully understood and varies among viruses, viral fusion typically involves specific fusagenic agents such as viral proteins, viral spike glycoproteins and peptides of viral spike glycoproteins. Vesicular stomatitis virus (VSV) fusion, for example, is thought to involve interaction between the VSV glycoprotein (G protein) and membrane lipids (Schlegel, R. et al. (1983) Cell 32:639–646). The VSV G protein reportedly binds preferentially to saturable receptors such as acidic phospholipid phosphatidylserine (Schlegel, R. and M. Wade (1985) J. Virol. 53(1):319–323). Fusion of influenza virus involves hemagglutinin HA-2N-terminal fusagenic peptides. See Kamata, H. et al. (1994) Nucl. Acids Res. 22(3):536–537.

Cell binding can also be enhanced or accelerated with peptides that bind cell receptors. For example, the penton-base protein of the Adenovirus coat contains the peptide motif RGD (Arg-Gly-Asp) which mediates binding to integrins and vira internalization via receptor-mediated endocytosis (Wickham, T. J. et al. (1995) Gene Therapy 2:750–756).

The efficiency of cationic lipid transfections has recently been shown to be enhanced by the addition of whole virus particles to the transfection mixture. See Yoshimura et al. (1993) J. Biol. Chem. 268:2300. Certain viral components may also enhance the efficiency of cationic lipid-mediated transfection. See: U.S. patent applications Ser. Nos. 08/090,290, filed Jul. 12, 1993, and 08/274,397, filed Jul. 12, 1994, incorporated by reference in their entirety herein. The use of peptides from viral proteins to enhance lipid-mediated transfections was also recently suggested by Kamata et al. (1994) Nucl. Acids Res. 22:536. Kamata et al. suggest that "LIPOFECTIN"-mediated transfections may be enhanced 3-4-fold by adding influenza virus hemagglutinin peptides to the transfection mixture. Despite these positive early indications, results vary as to the effectiveness of including fusagenic peptides in lipidic transfection compositions. Remy et al. (1995) Proc. Natl. Acad. Sci. U.S.A. 92:1744 report that "[a]ddition of lipids bearing a fusagenic or a nuclear localization peptide head group to the (polycationic lipid-DNA complex) particles does not significantly improve an already efficient system."

The present invention is based on the discovery that peptide sequences from viral proteins can significantly enhance the efficiency of cationic lipid-mediated transfection of eukaryotic cells. The compositions and methods of the invention comprise fusagenic receptor-ligand, or nuclear localization peptides which significantly improve the efficiency of transfection when bound to nucleic acid prior to adding the transfection reagent. These fusagenic, receptor-ligand, and nuclear localization peptides form a noncovalent association or complex with the DNA. Complex formation may be enhanced by covalently coupling the peptide to a DNA binding group, which binds to the nucleic acid through conformational or charge interactions between the binding group and the DNA. These bound nucleic acids are more efficiently transported into the cell and to the cell nucleus, thus requiring less nucleic acid starting material. The cationic lipid compositions of the present invention provide